REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Plant genes that protect against herbivorous insects may be useful for heterologous expression in food and fiber crops. Proteinase inhibitors (PIs) are common in plants and have drawn attention as possible transgenes for insect defense in crops. PIs are of particular interest because they are generally the product of a single gene, and inhibit proteolytic enzymes of animal and fungal origin, but rarely plant origin, and therefore are thought to act as protective agents. Several studies have demonstrated that PIs might provide adequate protection against a variety of economically important lepidopteran insects.

The production and accumulation of PIs in plants can be activated by a variety of mechanisms. Potato, tomato, and poplar PIs have been shown to be wound inducible, both at the site of wounding and systemically. In contrast, the production of PIs in cabbage (*Brassica oleracea*), especially trypsin and chymotrypsin inhibitors, are linked to plant development. Low levels of PI activity in cabbage are produced in young foliage in seedlings. When the plant reaches the 11-13 leaf stage, the level of PI activity gradually increases in young leaves, and reaches a maximal level of activity in the young foliage on mature plants. The production of PIs in cabbage is synchronized with the appearance of herbivorous insects in the field. Thus, the PIs are present when the resistance factor is most needed against these pests. In addition, cabbage foliar extracts containing PIs have been shown to significantly reduce growth and development of larval Lepidoptera and plant pathogenic fungi.

Genetic engineering of plants, which entails the isolation and manipulation of genetic material (usually in the form of DNA or RNA), and the subsequent introduction of that genetic material into plants or plant cells, offers considerable promise as a tool for the control of plant pests. If transgenic plants can be developed which express naturally occurring pest inhibitors, the need for expensive and potentially harmful chemical pest control measures is reduced. What is needed is a method of providing, and/or enhancing protection against herbivorous insects through the expression of the cabbage PI in crop plants. The present invention is directed to overcoming these and other deficiencies in the art.

The objections to claims 1, 2, 9-13, and 15-20 are respectfully traversed in view of the above amendments.

The rejection of claims 1-13 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 1-20 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed.

It is the position of the U.S. Patent and Trademark Office ("PTO") that while enabling for isolated nucleic acids encoding the polypeptide of SEQ ID NO: 2 from cabbage, and transgenic plant and seeds comprising that nucleic acid, the specification does not reasonably provide enablement for any nucleic acid molecule that hybridizes to SEQ ID NO: 1 under the specified conditions and encoding a protein having the desired activity, or transgenic plants and seeds transformed with said nucleic acid. Applicants respectfully disagree.

Applicants submit that the claimed invention is fully enabled by the present application. Moreover, as demonstrated below, based on the accompanying Declaration of C. Neal Stewart, Jr., Under 37 C.F.R. § 1.132 ("Stewart Declaration"), as well as the exhibits referenced therein, the disclosure of the present application would have enabled a skilled scientist to prepare additional nucleic acid constructs having a nucleic acid molecule from *Brassica oleracea* encoding a Kunitz-type serine proteinase inhibitor and to use such constructs to confer insect resistance to plants by transforming plants with these constructs.

The PTO cites Broun et al., "Catalytic Plasticity of Fatty Acid Modification Enzymes Underlying Chemical Diversity of Plant Lipids," *Science* 282:1315-1317 (1998) ("Broun") and Lazar et al., "Transforming Growth Factor α: Mutation of Aspartic Acid 47 and Leucine 48 Results in Different Biological Activities," *Mol. Cell Biol.* 8(3):1247-1252 (1988) ("Lazar") to support the position that the state of the prior art teaches that not all nucleotide sequences that hybridize to each other will encode proteins of similar function, even if the hybridization conditions are of relatively high stringency. However, neither Broun nor Lazar are applicable to the claimed invention.

Lazar teaches that mutation of two amino acids that are conserved in the family of the EGF-like peptides and are located in the carboxy-terminal part of TGF-α resulted in different effects on the biological activity of the two peptides. Broun teaches that as few as four amino acid substitutions can convert an oleate 12-desaturase to a hydroxylase, and as few as six amino acid substitutions result in conversion of a hydroxylase to a desaturase. First, the PTO has not provided any evidence that the peptides of Lazar, which are growth factors, and the peptides of Broun, which are enzymes involved in fatty acid synthesis, have any functional or structural relationship to the claimed nucleic acid construct

containing one or more nucleic acid molecules encoding a Kunitz-type serine proteinase inhibitor. Accordingly, there is no basis to apply the teachings of these publications to the claimed invention and conclude that the phenomena noted in Lazar and Broun would necessarily occur in the proteinase inhibitors of the present invention if there was a mutation or substitution of a few amino acids. Furthermore, the literature teaches that such a conclusion is, in fact, not applicable to the proteins of the present invention.

Specifically, proteins that function as proteinase inhibitors are exceptional among proteins, because they tend to retain their inhibitory activity even when the active site residue ("P_1") is replaced by another residue (Stewart Declaration ¶ 6). Unlike other proteins, in which the replacement of active site residues leads to a complete loss or dramatic decrease in activity, proteinase inhibitors have the ability to tolerate a synthetic or mutational replacement of the P_1 residue (Id.). In some cases, such a substitution leads to a predictable change in inhibitory activity, e.g., an Arg63 to Trp63 substitution at the active site in the archetypal Kunitz soybean trypsin inhibitor ("STI") leads to the conversion of a trypsin inhibitor to a chymotrypsin inhibitor (Id.). Thus, within each inhibitor family, the P_1 residue is not conserved, but changes frequently, resulting in inhibitory specificity, but not in a loss of biological activity (Id.). For example, it has been shown that generally, inhibitors with P_1 Lys and Arg tend to inhibit trypsin and trypsin-like enzymes, those with P_1 Tyr, Phe, Trp, Leu, and Met inhibit chymotrypsin and chymotrypsin-like enzymes, and those with P_1 Ala and Ser inhibit-elastase-like enzymes (Id.). Because "serine proteinase inhibitor" encompasses inhibitors of trypsin, chymotrypsin, and elastase (see Example 9), a mutation or substitution in even the active site residue of a serine proteinase inhibitor can still result in a protein with serine proteinase inhibitory activity (Id.)

Therefore, applicants submit that a nucleic acid molecule that hybridizes to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1 of the present application under stringent conditions may encode a protein with some variation in amino acid sequence from the protein encoded by SEQ ID NO: 1 while still retaining functionality as a serine proteinase inhibitor (Stewart Declaration ¶ 7). Furthermore, a skilled scientist would not expect substantial variation among species encompassed by the claimed invention because of the highly stringent conditions set forth in the claims, some, but not much, variation would be expected in either the nucleotide sequence or the protein it encodes (Id.). Thus, applicants submit that one of ordinary skill in the art could make and use other nucleic acid molecules from *Brassica oleracea* that encode additional serine proteinases, based on

the disclosure of the present application and the prior art knowledge that active site residues vary among the serine proteinase inhibitors and identify their inhibitory specificity.

Furthermore, applicants submit that the present application teaches with particularity how to make and use a nucleic acid molecule that hybridizes to the nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1 under stringent conditions (Stewart Declaration ¶ 8).

Specifically, the present application teaches the isolation of a PI gene from plant material and the preparation of a cDNA library having a PI clone (Example 1), the preparation of a shuttle vector containing the PI gene (Example 2), the transfer of the gene into a plant expression vector construct and subsequent transformation into *Agrobacterium tumefaciens* for use in transforming plant cultures (Example 3), and the characterization of the *bopi* gene, including the open reading frame of 642 bp, which encodes the mature BOPI protein (Example 4) (Stewart Declaration ¶ 9). The BoPI protein is highly characterized in the present application at Example 5 (Id.). The amino acid sequence of the protein is disclosed, a putative Kunitz inhibitor family signature is identified, the Arginine active site residue at position 63 is disclosed, and the four cysteines of the expected disulfide bonds are identified (pg. 23, lines 5-28) (Id.). Furthermore, the BoPI protein is characterized as having a molecular weight of 21 kDa and an isoelectric point of 4.94 (pg. 8, lines 25-29) (Id.).

In addition, Example 6 teaches that the PI gene isolated from *Brassica oleracea* is one of a family of PI genes, thereby teaching that additional PI genes may be isolated from *Brassica oleracea* (Stewart Declaration ¶ 10). Examples 7-10 teach how to make heterologous transgenic plants having a PI gene of the present invention, and provide methods for determining the specificity and efficacy of the proteinase inhibitor's insect antibiosis activity when a serine proteinase inhibitor is expressed in a transgenic plant (Id.).

Therefore, it is clear that a skilled scientist having read the present application would know: (1) how to make additional nucleic acid constructs having one or more operatively linked nucleic acid molecules which encode a Kunitz-type serine proteinase inhibitor isolated from *Brassica oleracea* having insect antibiosis activity, an operably linked heterologous DNA promoter, and an operably linked 3' regulatory region; (2) how to use such constructs to prepare expression vectors and host cells, including plant cells; and (3) how to prepare transgenic plants transformed with the construct that are resistant to insects (Stewart Declaration ¶ 11).

Thus, applicants submit that the present invention is fully enabled as filed, and therefore, the rejection of claims 1-20 under 35 U.S.C. § 112 (1st para.) for lack of enablement is improper and should be withdrawn.

The rejection of claims 1-20 under 35 U.S.C. § 112 (1st para.) for failure to meet the written description requirement is respectfully traversed.

As amended, claim 1 is drawn to "[a] nucleic acid construct comprising: one or more operatively linked nucleic acid molecules, wherein the nucleic acid molecule encodes a Kunitz-type serine proteinase inhibitor isolated from *Brassica oleracea,* wherein the serine proteinase inhibitor has insect antibiosis activity; and having an operably linked heterologous DNA promoter; and an operably linked 3' regulatory region." Applicants submit that one skilled in the art, having read the present application, would recognize that the applicants were in possession of the invention as claimed.

The PTO has taken the position that claim 1 is drawn to a nucleic acid molecule encoding a serine protease inhibitor from *Brassica oleracea* described by function only, and, for that reason, applicants have failed to meet the written description requirement. This rejection is proper only if the name of the claimed composition does no more than distinguish the claimed genus from all others by function, without defining any structural features commonly possessed by members of the genus that distinguish them from others (Guidelines for Examination of Patent Applications under the 35 U.S.C. 112 ¶1 "Written Description Requirement," Fed. Reg. Vol. 66, No. 4, January 5, 2001, Notices). Applicants submit that the recitation of "a Kunitz-type serine proteinase inhibitor" in claim 1 as amended distinguishes the claimed subject matter with functional and structural specificity. Moreover, as demonstrated below, based on the accompanying Declaration of C. Neal Stewart, Jr., Under 37 C.F.R. § 1.132 ("Stewart Declaration"), as well as the exhibits referenced therein, a skilled scientist, having read the present application, would recognize that the applicants were in possession of the invention as claimed.

In particular, protease inhibitors ("PIs") from plants have been studied for over 50 years (Stewart Declaration ¶ 12). PIs are categorized into families, based on analogy (similar function), homology (similar amino acid structure), and mechanism of inhibiting proteinase activity (Id.). It is known that most individual protein inhibitors inhibit proteinases belonging to a single mechanistic class (Id.). Of these, the inhibitors of serine proteinases are the most studied (Id.) and, thus, are the most highly characterized (Id.). Sequencing and X-ray crystallography have shown that these inhibitors are not all homologous; rather they belong to about 10 homologous families (Id.). As disclosed in the

present application, the proteinase inhibitors of the present invention belong to the Kunitz family of proteinase inhibitors (see Example 5) (Id.). Kunitz-type proteins are characterized as having a molecular weight (M_r) of about 21,000-22,000 Daltons, two-disulfide bonds (4-half-cystine residues) and a single reactive site for serine proteases (Id.).

Another characteristic which is used to establish PI families are the topological relationships between the disulfide bonds (also known as disulfide "bridges") and the location of the PI's reactive site (Stewart Declaration ¶ 13). In each family, the positions of all intrachain disulfide bridges are completely conserved (Id.). Furthermore, recent X-ray crystallographic studies of the archetypal member of the Kunitz family, soybean trypsin inhibitor ("STI"), show that Kunitz-type inhibitors from a variety of plant sources share a high degree of homology to the three dimensional structure of STI (Id.). Using the studies based on the structure of STI, it can be predicted that the reactive site of the serine proteinase inhibitors of the present invention is positioned on an exposed loop of a characteristic canonical confirmation, unconstrained by secondary structural elements or disulfide bridges that could limit its conformational freedom (Id.). It has been reported that in Kunitz-type mono- and dicotyledons there is clear alignment of the P_1 sites on the loop whether for trypsin, chymotrypsin or subtilisin (Id.). In addition, proteinase inhibitors from *Brassica oleracea* have been characterized as having molecular weights ranging from 9-25 kDa, isoelectric points from 4.5-5.0, and trypsin and chymotrypsin activity that is relatively stable over a range of temperature from 0-100°C and at pH values of 4.5-7.5 (Id.).

Applicants submit that all of the above information defines the claimed invention structurally as well as functionally and, furthermore, provides a distinct relationship between the structure and the function of the claimed subject matter. With this information, one of ordinary skill in the art, having read the present application, would understand that at the time the application was filed, applicants were in possession of the invention as claimed.

The PTO has cited W0 91/09060 to Broadway ("Broadway II") to support the position that the structural characteristic of an inhibitor must be known before the inhibitor can be used against specific pests. Applicants disagree.

Broadway II suggests that the efficacy of a specific inhibitor from an individual plant is dependent upon 1) the unique structure of the plant proteinase inhibitor, and 2) the susceptibility of the proteinase in the target organism (see page 2, lines 19-24) (Stewart Declaration ¶ 14). Applicants submit that while the efficacy of an inhibitor may be dependent, in part, on the inhibitor's unique structure, one can utilize an inhibitor against a susceptible target organism (i.e., a herbivorous insect) without knowledge of the inhibitor's

structure because the inhibitor exhibits its functional activity against a susceptible target whether the user is cognizant of the structure of the inhibitor or not (Id.). For example, Broadway II used three PIs isolated from cabbage to successfully inhibit the growth of herbivorous insects, knowing nothing more about the proteins than their molecular weight, isoelectric point, and enzymatic inhibitory activity (Id.). It was the knowledge of the specific activity of a proteinase inhibitor that drove Broadway's choice to use a particular PI against a given insect. A skilled scientist would not consider it undue experimentation to test the specific activity of a serine PI (Id.). This can be done by carrying out an enzyme inhibition assay against trypsin, chymotrypsin, or elastase, such as described in the present application (see Example 9) or as disclosed in art, or by using a bioassay (Id.). Once the specificity of a molecule is identified, the molecule can be used as taught in the present application to confer insect resistance to plants. Thus, applicants submit that a gene, including one encoding a serine proteinase inhibitor, and the protein it encodes, can be used successfully for its intended purpose without a complete understanding of the structural character of either the nucleic acid molecule or the protein (Id.).

Finally, because the chemical and structural profiles of the Kunitz-type serine proteinase inhibitors are so well-characterized in the literature, applicants submit that it was not necessary to reiterate those details in the specification. "What is conventional or well known to one in the art need not be disclosed in detail. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described on the specification, then the adequate description requirement is met." Methodology for Determining Adequacy of Written Description, *Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶1, Written Description Requirement* 66 Fed. Reg. 1099, 1106 (2001).

Thus, the specification describes the claimed invention in sufficiently clear and concise terms that one of ordinary skill in the art, having read the present application, would understand that applicants were in possession of the invention as claimed.

Therefore, the rejection of claims 1-20 under 35 U.S.C. § 112 (1st para.) for failure to meet the written description requirement is improper and should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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